

## **Restriction of PCR product**

This is a standard BioBrick restriction of PCR product containing a BioBrick suffix and prefix. These standard restrictions are performed using EcoRI, PstI, XbaI, SpeI, NdeI, all high fidelity restriction enzymes.

### *Materials*

- CutSmart buffer
- RNase/DNase free water
- Restriction enzymes

### *Method for analysis*

1. Use 25 µl PCR product for each restriction.
2. Add 1/10 of the total restriction volume of CutSmart buffer.
3. Use 0.5 µl of each 20.000 units restriction enzyme. (Use 1 µl of 10.000 units restriction enzymes.)
4. Add RNase/DNase free water up to the total restriction volume.
5. Mix gently and incubate in a waterbath at 37 C for 3 hour.
6. Go to the protocol gel electrophoresis.

### *Method for ligation*

1. Use 25 µl PCR product for each restriction.
2. Add 1/10 of the total restriction volume of CutSmart buffer.
3. Use 0.5 µl of each 20.000 units restriction enzyme. (Use 1 µl of 10.000 units restriction enzymes.)
4. Add RNase/DNase free water up to the total restriction volume.
5. Mix gently and incubate in a waterbath at 37 C for 3 hour.